

SEQUENCE LISTING

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<110> Steiger, Sabine
Sandmann, Gerhard

<120> Method for producing ketocarotenoids by cultivating genetically
modified organisms

<130> 12810-00106-US

<150> PCT/EP2003/014876

<151> 2003-12-24

<150> DE 103 00 649.4

<151> 2003-01-09

<160> 15

<170> PatentIn version 3.3

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<211> 789

<212> DNA

<213> Nostoc sp. PCC73102

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Leu Ser Ala Lys Glu Asp Thr Val Trp Gly Leu Val Ile Val Ile Val	
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Ile Ile Ser Leu Trp Val Ala Ser Leu Ala Phe Leu Leu Ala Ile Asn	
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tat gcc aaa gtc cca att tgg ttg ata cct att gca ata gtt tgg caa	192
Tyr Ala Lys Val Pro Ile Trp Leu Ile Pro Ile Ala Ile Val Trp Gln	
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atg ttc ctt tat aca ggg cta ttt att act gca cat gat gct atg cat	240
Met Phe Leu Tyr Thr Gly Leu Phe Ile Thr Ala His Asp Ala Met His	
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Gly Ser Val Tyr Arg Lys Asn Pro Lys Ile Asn Asn Phe Ile Gly Ser	
85 90 95	

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 Phe His Asp Gly Lys Arg Thr Asn Ala Ile Phe Trp Tyr Leu His Phe
 130 135 140

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 Met Ile Glu Tyr Ser Ser Trp Gln Gln Leu Ile Val Leu Thr Ile Leu
 145 150 155 160

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 Phe Asn Leu Ala Lys Tyr Val Leu His Ile His Gln Ile Asn Leu Ile
 165 170 175

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 180 185 190

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 Phe Gly Thr Phe Leu Pro His Arg Glu Pro Lys Lys Gly Tyr Val Tyr
 195 200 205

ccc cat tgc agc caa aca ata aaa ttg cca act ttt ttg tca ttt atc 672
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 210 215 220

gct tgc tac cac ttt ggt tat cat gaa gaa cat cat gag tat ccc cat 720
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 225 230 235 240

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 35 40 45

Tyr Ala Lys Val Pro Ile Trp Leu Ile Pro Ile Ala Ile Val Trp Gln
 50 55 60

Met Phe Leu Tyr Thr Gly Leu Phe Ile Thr Ala His Asp Ala Met His
 65 70 75 80

Gly Ser Val Tyr Arg Lys Asn Pro Lys Ile Asn Asn Phe Ile Gly Ser
 85 90 95

Leu Ala Val Ala Leu Tyr Ala Val Phe Pro Tyr Gln Gln Met Leu Lys
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Asn His Cys Leu His His Arg His Pro Ala Ser Glu Val Asp Pro Asp
 115 120 125

Phe His Asp Gly Lys Arg Thr Asn Ala Ile Phe Trp Tyr Leu His Phe
 130 135 140

Met Ile Glu Tyr Ser Ser Trp Gln Gln Leu Ile Val Leu Thr Ile Leu
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Phe Asn Leu Ala Lys Tyr Val Leu His Ile His Gln Ile Asn Leu Ile
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Leu Phe Trp Ser Ile Pro Pro Ile Leu Ser Ser Ile Gln Leu Phe Tyr
 180 185 190

Phe Gly Thr Phe Leu Pro His Arg Glu Pro Lys Lys Gly Tyr Val Tyr
 195 200 205

Pro His Cys Ser Gln Thr Ile Lys Leu Pro Thr Phe Leu Ser Phe Ile
 210 215 220

Ala Cys Tyr His Phe Gly Tyr His Glu Glu His His Glu Tyr Pro His
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Val Leu Arg Ser Lys Ser Gln Phe Lys Gly Leu Phe Ile Ala Ile Val
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Ile Val Ser Ala Trp Val Ile Ser Leu Ser Leu Leu Leu Ser Leu Asp
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Ile Ser Lys Leu Lys Phe Trp Met Leu Leu Pro Val Ile Leu Trp Gln
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Thr Phe Leu Tyr Thr Gly Leu Phe Ile Thr Ser His Asp Ala Met His
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Gly Val Val Phe Pro Gln Asn Thr Lys Ile Asn His Leu Ile Gly Thr
85 90 95
ttg acc cta tcc ctt tat ggt ctt tta cca tat caa aaa cta ttg aaa 336
Leu Thr Leu Ser Leu Tyr Gly Leu Leu Pro Tyr Gln Lys Leu Leu Lys
100 105 110
aaa cat tgg tta cac cac cac aat cca gca agc tca ata gac ccg gat 384
Lys His Trp Leu His His His Asn Pro Ala Ser Ser Ile Asp Pro Asp
115 120 125
ttt cac aat ggt aaa cac caa agt ttc ttt gct tgg tat ttt cat ttt 432
Phe His Asn Gly Lys His Gln Ser Phe Phe Ala Trp Tyr Phe His Phe
130 135 140
atg aaa ggt tac tgg agt tgg ggg caa ata att gcg ttg act att att 480
Met Lys Gly Tyr Trp Ser Trp Gly Gln Ile Ile Ala Leu Thr Ile Ile
145 150 155 160
tat aac ttt gct aaa tac ata ctc cat atc cca agt gat aat cta act 528
Tyr Asn Phe Ala Lys Tyr Ile Leu His Ile Pro Ser Asp Asn Leu Thr

165

170

175

tac ttt tgg gtg cta ccc tcg ctt tta agt tca tta caa tta ttc tat 576
 Tyr Phe Trp Val Leu Pro Ser Leu Leu Ser Ser Leu Gln Leu Phe Tyr
 180 185 190

ttt ggt act ttt tta ccc cat agt gaa cca ata ggg ggt tat gtt cag 624
 Phe Gly Thr Phe Leu Pro His Ser Glu Pro Ile Gly Gly Tyr Val Gln
 195 200 205

cct cat tgt gcc caa aca att agc cgt cct att tgg tgg tca ttt atc 672
 Pro His Cys Ala Gln Thr Ile Ser Arg Pro Ile Trp Trp Ser Phe Ile
 210 215 220

acg tgc tat cat ttt ggc tac cac gag gaa cat cac gaa tat cct cat 720
 Thr Cys Tyr His Phe Gly Tyr His Glu Glu His His Glu Tyr Pro His
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<213> Nostoc sp. PCC73102

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Ile Val Ser Ala Trp Val Ile Ser Leu Ser Leu Leu Leu Ser Leu Asp
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Ile Ser Lys Leu Lys Phe Trp Met Leu Leu Pro Val Ile Leu Trp Gln
 50 55 60

Thr Phe Leu Tyr Thr Gly Leu Phe Ile Thr Ser His Asp Ala Met His
 65 70 75 80

Gly Val Val Phe Pro Gln Asn Thr Lys Ile Asn His Leu Ile Gly Thr
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Leu Thr Leu Ser Leu Tyr Gly Leu Leu Pro Tyr Gln Lys Leu Leu Lys
 100 105 110

Lys His Trp Leu His His His Asn Pro Ala Ser Ser Ile Asp Pro Asp
 115 120 125

Phe His Asn Gly Lys His Gln Ser Phe Phe Ala Trp Tyr Phe His Phe
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Met Lys Gly Tyr Trp Ser Trp Gly Gln Ile Ile Ala Leu Thr Ile Ile
 145 150 155 160

Tyr Asn Phe Ala Lys Tyr Ile Leu His Ile Pro Ser Asp Asn Leu Thr
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Tyr Phe Trp Val Leu Pro Ser Leu Leu Ser Ser Leu Gln Leu Phe Tyr
 180 185 190

Phe Gly Thr Phe Leu Pro His Ser Glu Pro Ile Gly Gly Tyr Val Gln
 195 200 205

Pro His Cys Ala Gln Thr Ile Ser Arg Pro Ile Trp Trp Ser Phe Ile
 210 215 220

Thr Cys Tyr His Phe Gly Tyr His Glu Glu His His Glu Tyr Pro His
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ggc cca cct cct cat ctc cat cgg tca ttt gct gct acc acg atg ctg 95
 Gly Pro Pro Pro His Leu His Arg Ser Phe Ala Ala Thr Thr Met Leu
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 Ser Lys Leu Gln Ser Ile Ser Val Lys Ala Arg Arg Val Glu Leu Ala

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acc gtg cag gct gcc ggc gcg ggc gat gag cac agc gcc gat gta gca Thr Val Gln Ala Ala Gly Ala Gly Asp Glu His Ser Ala Asp Val Ala 80 85 90 95			287
ctc cag cag ctt gac cgg gct atc gca gag cgt cgt gcc cgg cgc aaa Leu Gln Gln Leu Asp Arg Ala Ile Ala Glu Arg Arg Ala Arg Arg Lys 100 105 110			335
cgg gag cag ctg tca tac cag gct gcc gcc att gca gca tca att ggc Arg Glu Gln Leu Ser Tyr Gln Ala Ala Ala Ile Ala Ala Ser Ile Gly 115 120 125			383
gtg tca ggc att gcc atc ttc gcc acc tac ctg aga ttt gcc atg cac Val Ser Gly Ile Ala Ile Phe Ala Thr Tyr Leu Arg Phe Ala Met His 130 135 140			431
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ctc ttg gtg gtt ggt ggc gcg ctc ggc atg gag atg tat gcc cgc tat Leu Leu Val Val Gly Gly Ala Leu Gly Met Glu Met Tyr Ala Arg Tyr 160 165 170 175			527
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65 70 75 80

Val Gln Ala Ala Gly Ala Gly Asp Glu His Ser Ala Asp Val Ala Leu
85 90 95

Gln Gln Leu Asp Arg Ala Ile Ala Glu Arg Arg Ala Arg Arg Lys Arg
100 105 110

Glu Gln Leu Ser Tyr Gln Ala Ala Ala Ile Ala Ala Ser Ile Gly Val
115 120 125

Ser Gly Ile Ala Ile Phe Ala Thr Tyr Leu Arg Phe Ala Met His Met
130 135 140

Thr Val Gly Gly Ala Val Pro Trp Gly Glu Val Ala Gly Thr Leu Leu
145 150 155 160

Leu Val Val Gly Gly Ala Leu Gly Met Glu Met Tyr Ala Arg Tyr Ala
165 170 175

His Lys Ala Ile Trp His Glu Ser Pro Leu Gly Trp Leu Leu His Lys
180 185 190

Ser His His Thr Pro Arg Thr Gly Pro Phe Glu Ala Asn Asp Leu Phe
195 200 205

Ala Ile Ile Asn Gly Leu Pro Ala Met Leu Leu Cys Thr Phe Gly Phe
210 215 220

Trp Leu Pro Asn Val Leu Gly Ala Ala Cys Phe Gly Ala Gly Leu Gly
225 230 235 240

Ile Thr Leu Tyr Gly Met Ala Tyr Met Phe Val His Asp Gly Leu Val
245 250 255

His Arg Arg Phe Pro Thr Gly Pro Ile Ala Gly Leu Pro Tyr Met Lys
260 265 270

Arg Leu Thr Val Ala His Gln Leu His His Ser Gly Lys Tyr Gly Gly

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280

285

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 Met Asp
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act ttg ttg aaa acc cca aat aac ctt gaa ttt ctg aac cca cat cat 165
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ggt ttt gct gtt aaa gct agt acc ttt aga tct gag aag cat cat aat 213
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ttt ggt tct agg aag ttt tgt gaa act ttg ggt aga agt gtt tgt gtt 261
 Phe Gly Ser Arg Lys Phe Cys Glu Thr Leu Gly Arg Ser Val Cys Val
 35 40 45 50

aag ggt agt agt agt gct ctt tta gag ctt gta cct gag acc aaa aag 309
 Lys Gly Ser Ser Ser Ala Leu Leu Glu Leu Val Pro Glu Thr Lys Lys
 55 60 65

gag aat ctt gat ttt gag ctt cct atg tat gac cct tca aaa ggg gtt 357
 Glu Asn Leu Asp Phe Glu Leu Pro Met Tyr Asp Pro Ser Lys Gly Val
 70 75 80

gtt gtg gat ctt gct gtg gtt ggt ggt ggc cct gca gga ctt gct gtt 405
 Val Val Asp Leu Ala Val Val Gly Gly Gly Pro Ala Gly Leu Ala Val
 85 90 95

gca cag caa gtt tct gaa gca gga ctc tct gtt tgt tca att gat ccg 453

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Phe	Glu	Ala	Met	Asp	Leu	Leu	Asp	Cys	Leu	Asp	Ala	Thr	Trp	Ser	Gly	
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gca	gca	gtg	tac	att	gat	gat	aat	acg	gct	aaa	gat	ctt	cat	aga	cct	597
Ala	Ala	Val	Tyr	Ile	Asp	Asp	Asn	Thr	Ala	Lys	Asp	Leu	His	Arg	Pro	
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Tyr	Gly	Arg	Val	Asn	Arg	Lys	Gln	Leu	Lys	Ser	Lys	Met	Met	Gln	Lys	
		165					170					175				
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Cys	Ile	Met	Asn	Gly	Val	Lys	Phe	His	Gln	Ala	Lys	Val	Ile	Lys	Val	
	180					185					190					
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Ile	His	Glu	Glu	Ser	Lys	Ser	Met	Leu	Ile	Cys	Asn	Asp	Gly	Ile	Thr	
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Val	Gln	Tyr	Asp	Lys	Pro	Tyr	Asn	Pro	Gly	Tyr	Gln	Val	Ala	Tyr	Gly	
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Ile	Leu	Ala	Glu	Val	Glu	Glu	His	Pro	Phe	Asp	Val	Asn	Lys	Met	Val	
		245					250					255				
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Phe	Met	Asp	Trp	Arg	Asp	Ser	His	Leu	Lys	Asn	Asn	Thr	Asp	Leu	Lys	
	260					265					270					
gag	aga	aat	agt	aga	ata	cca	act	ttt	ctt	tat	gca	atg	cca	ttt	tca	981
Glu	Arg	Asn	Ser	Arg	Ile	Pro	Thr	Phe	Leu	Tyr	Ala	Met	Pro	Phe	Ser	
275					280					285					290	
tcc	aac	agg	ata	ttt	ctt	gaa	gaa	aca	tca	ctc	gta	gct	cgt	cct	ggc	1029
Ser	Asn	Arg	Ile	Phe	Leu	Glu	Glu	Thr	Ser	Leu	Val	Ala	Arg	Pro	Gly	
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Leu	Arg	Ile	Asp	Asp	Ile	Gln	Glu	Arg	Met	Val	Ala	Arg	Leu	Asn	His	
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Leu	Gly	Ile	Lys	Val	Lys	Ser	Ile	Glu	Glu	Asp	Glu	His	Cys	Leu	Ile	

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Pro Met Gly Gly Pro Leu Pro Val Leu Pro Gln Arg Val Val Gly Ile			
340	345	350	
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Gly Gly Thr Ala Gly Met Val His Pro Ser Thr Gly Tyr Met Val Ala			
355	360	365	370
agg aca cta gct gcg gct cct gtt gtt gcc aat gcc ata att caa tac			1269
Arg Thr Leu Ala Ala Ala Pro Val Val Ala Asn Ala Ile Ile Gln Tyr			
375	380	385	
ctc ggt tct gaa aga agt cat tcg ggt aat gaa tta tcc aca gct gtt			1317
Leu Gly Ser Glu Arg Ser His Ser Gly Asn Glu Leu Ser Thr Ala Val			
390	395	400	
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Trp Lys Asp Leu Trp Pro Ile Glu Arg Arg Arg Gln Arg Glu Phe Phe			
405	410	415	
tgc ttc ggt atg gat att ctt ctg aag ctt gat tta cct gct aca aga			1413
Cys Phe Gly Met Asp Ile Leu Leu Lys Leu Asp Leu Pro Ala Thr Arg			
420	425	430	
agg ttc ttt gat gca ttc ttt gac tta gaa cct cgt tat tgg cat ggc			1461
Arg Phe Phe Asp Ala Phe Phe Asp Leu Glu Pro Arg Tyr Trp His Gly			
435	440	445	450
ttc tta tcg tct cga ttg ttt cta cct gaa ctc ata gtt ttt ggg ctg			1509
Phe Leu Ser Ser Arg Leu Phe Leu Pro Glu Leu Ile Val Phe Gly Leu			
455	460	465	
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Ser Leu Phe Ser His Ala Ser Asn Thr Ser Arg Phe Glu Ile Met Thr			
470	475	480	
aag gga act gtt cca tta gta aat atg atc aac aat ttg tta cag gat			1605
Lys Gly Thr Val Pro Leu Val Asn Met Ile Asn Asn Leu Leu Gln Asp			
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Lys Glu			
500			

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His Asn Phe Gly Ser Arg Lys Phe Cys Glu Thr Leu Gly Arg Ser Val
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65 70 75 80

Gly Val Val Val Asp Leu Ala Val Val Gly Gly Gly Pro Ala Gly Leu
85 90 95

Ala Val Ala Gln Gln Val Ser Glu Ala Gly Leu Ser Val Cys Ser Ile
100 105 110

Asp Pro Asn Pro Lys Leu Ile Trp Pro Asn Asn Tyr Gly Val Trp Val
115 120 125

Asp Glu Phe Glu Ala Met Asp Leu Leu Asp Cys Leu Asp Ala Thr Trp
130 135 140

Ser Gly Ala Ala Val Tyr Ile Asp Asp Asn Thr Ala Lys Asp Leu His
145 150 155 160

Arg Pro Tyr Gly Arg Val Asn Arg Lys Gln Leu Lys Ser Lys Met Met
165 170 175

Gln Lys Cys Ile Met Asn Gly Val Lys Phe His Gln Ala Lys Val Ile
180 185 190

Lys Val Ile His Glu Glu Ser Lys Ser Met Leu Ile Cys Asn Asp Gly
195 200 205

Ile Thr Ile Gln Ala Thr Val Val Leu Asp Ala Thr Gly Phe Ser Arg
210 215 220

Ser Leu Val Gln Tyr Asp Lys Pro Tyr Asn Pro Gly Tyr Gln Val Ala
225 230 235 240

Tyr Gly Ile Leu Ala Glu Val Glu Glu His Pro Phe Asp Val Asn Lys
 245 250 255

Met Val Phe Met Asp Trp Arg Asp Ser His Leu Lys Asn Asn Thr Asp
 260 265 270

Leu Lys Glu Arg Asn Ser Arg Ile Pro Thr Phe Leu Tyr Ala Met Pro
 275 280 285

Phe Ser Ser Asn Arg Ile Phe Leu Glu Glu Thr Ser Leu Val Ala Arg
 290 295 300

Pro Gly Leu Arg Ile Asp Asp Ile Gln Glu Arg Met Val Ala Arg Leu
 305 310 315 320

Asn His Leu Gly Ile Lys Val Lys Ser Ile Glu Glu Asp Glu His Cys
 325 330 335

Leu Ile Pro Met Gly Gly Pro Leu Pro Val Leu Pro Gln Arg Val Val
 340 345 350

Gly Ile Gly Gly Thr Ala Gly Met Val His Pro Ser Thr Gly Tyr Met
 355 360 365

Val Ala Arg Thr Leu Ala Ala Ala Pro Val Val Ala Asn Ala Ile Ile
 370 375 380

Gln Tyr Leu Gly Ser Glu Arg Ser His Ser Gly Asn Glu Leu Ser Thr
 385 390 395 400

Ala Val Trp Lys Asp Leu Trp Pro Ile Glu Arg Arg Arg Gln Arg Glu
 405 410 415

Phe Phe Cys Phe Gly Met Asp Ile Leu Leu Lys Leu Asp Leu Pro Ala
 420 425 430

Thr Arg Arg Phe Phe Asp Ala Phe Phe Asp Leu Glu Pro Arg Tyr Trp
 435 440 445

His Gly Phe Leu Ser Ser Arg Leu Phe Leu Pro Glu Leu Ile Val Phe
 450 455 460

Gly Leu Ser Leu Phe Ser His Ala Ser Asn Thr Ser Arg Phe Glu Ile

465

470

475

480

Met Thr Lys Gly Thr Val Pro Leu Val Asn Met Ile Asn Asn Leu Leu
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Gln Asp Lys Glu
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 <213> Artificial Sequence

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 <223> Primer: 148-Start

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22

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24

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26

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<212> DNA
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 <223> Primer: 38-End

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<220>
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 <223> pTP09

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 cttaaatacca atcccaatat caccacctcc cgccgccgta ctcttctctc cgccgccgcc 180
 gccgccgtcg taaggtcacc ggcgattcgt gcctcagctg caaccgaaac catagagaaa 240
 actgagactg cgggatcc 258

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 <211> 260
 <212> DNA
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 cttaaatacca atcccaatat caccacctcc cgccgccgta ctcttctctc cgccgccgcc 180
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 actgagactg cgctggatcc 260

<210> 15
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<220>
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cttaaatcca atcccaatat caccacctcc cgccgccgta ctcttctctc cgccgccgcc 180
gccgccgtcg taaggtcacc ggcgattcgt gcctcagctg caaccgaaac catagagaaa 240
actgagactg cggggatcc 259